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Impact of fungicides on *Metarhizium anisopliae* in the rhizosphere, bulk soil and in vitro

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Abstract The entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) is registered in the United States and The Netherlands for black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) control in container-grown ornamentals. These studies were conducted to determine the compatibility of *M. anisopliae* (F52) with a wide range of fungicides commonly applied to container-grown ornamentals for the management of soil-borne plant pathogens. The impact of fungicides on spore germination and mycelial growth were determined in vitro. In addition, *M. anisopliae* persistence in bulk and rhizosphere soil was determined 30 days following dual application of each fungicide at 7–28 days intervals as prescribed. A number of fungicides (thiophanate-methyl, dimethomorph, captan, triflumizole, triflozystrobin, pyraclostrobin, azoxystrobin) inhibited spore germination in vitro. A larger number of fungicides (fosetyl-AI, thiophanate-methyl, dimethomorph, captan, quintozene, triflumizole, fludioxanil, triflozystrobin, pyraclostrobin, fludiox-mefanox, iprodione, azoxystrobin, phosphorus acid/K-salts) inhibited mycelial growth in vitro. Only three fungicides (etridiazole, propamocard and mafanoxam) had no significant impact in vitro on spore germination or mycelial growth. While a number of fungicides had a detrimental impact in vitro, there was no impact on *M. anisopliae* populations in bulk soil following dual application of any fungicide. However, the fungicides captan and triflumizolet, which have a short reapplication interval, had a detrimental impact on *M. anisopliae* populations in the rhizosphere. As researchers develop rhizosphere competence as an alternative management strategy for black vine weevil, the fungicides captan and triflumizole should be avoided.

Keywords Black vine weevil · Ornamentals · Nursery · *Metarhizium anisopliae* · Rhizosphere soil · Soil applications · Fungicide · Side effects

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Introduction

The black vine weevil (BVW), *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) is a univoltine, polyphagous insect that is a serious pest of field and container-grown ornamentals as well as small fruit crops worldwide (Moorhouse et al. 1992). Adults are nocturnal and cause mainly cosmetic damage to plants by notching the leaves. Adults reproduce by thelytokous parthenogenesis so a single individual left unchecked can result in the infestation of an entire nursery. Oviposition occurs at night with eggs either dropped on the soil surface or inserted

into soil crevices (Smith 1932). Early instars begin feeding on small roots while the later instars feed on larger roots, especially on the phloem and cambium tissues near the soil surface (La Lone and Clarke 1981). The control program currently implemented by a majority of growers in the United States centers on the use of broad spectrum insecticides to target adults prior to oviposition. However, even when implementing an extensive spray program, growers often discover infested plant material. Infested plants cannot be sold and if infested plants are mistakenly shipped, the grower risks refusal by the buyer and will incur return shipping costs and potential loss of future sales. To help combat this difficult to manage pest, growers are escalating their use of soil incorporated insecticides.

Metarhizium anisopliae (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) has been studied extensively for the biological control of a wide range of insect pests, including BVW (Moorhouse et al. 1992, 1993a, b; Booth and Shanks 1998; Bruck 2005; Bruck and Donahue 2007). A recent tool available to nursery growers in the United States and The Netherlands for BVW management is the incorporation of *M. anisopliae* (F52) into media at potting (i.e., a plug in replacement for soil incorporated insecticides). This isolate persists well in commercial peat and bark-based potting media (Bruck 2005; Bruck and Donahue 2007), as well as in commonly used potting media components (coir, peat, hemlock bark, fir bark, perlite) (Bruck 2006). In addition to its efficacy as a soil incorporant, at least two isolates of *M. anisopliae* have been shown to be rhizosphere competent (Hu and St. Leger 2002; Bruck 2005). "Rhizosphere competence" has been defined when considering biological control agents as, "the ability of a microorganism, applied by seed treatment, to colonize the rhizosphere of developing roots" (Baker 1991). A definition for rhizosphere competent entomopathogenic fungi requires that the fungus grow and persist in the presence of natural flora in the soil or potting media and have the ability to colonize the rhizosphere and developing roots at populations great enough to infect pest insects feeding on the root. A larger *M. anisopliae* population in the inner rhizosphere compared with the outer rhizosphere suggests that response to root exudates is involved in the rhizosphere effect or that sporulation is enhanced in the rhizosphere (Hu and St. Leger 2002). Positive response to root exudates by *M. anisopliae* was also suggested by Klingen et al. (2002). Different isolates of the same entomopathogenic fungus can have varying pathogenicity for a particular pest (Poprawski et al. 1985; Bruck 2004) as well as respond differently to biotic and abiotic conditions. Biological control agents differ fundamentally from chemical agents in that in order to be effective, they must proliferate in the environment they are introduced (Nelson et al. 1994). These factors make it important to focus research on isolates that are commercially available and will be used in the field by growers to manage BVW populations.

The production of ornamental nursery plants is a complex process. Growers must concern themselves with a number of production hurdles when implementing their integrated management programs. In addition to insects, another serious production constraint is the presence of plant pathogens. Fungal plant pathogens are of particular concern because of the prophylactic measures needed to manage them. Because of this, there is a high probability that container-grown plants grown in media incorporated with *M. anisopliae* for BVW control, particularly in the US Pacific Northwest, will also be treated with one or more of a number of soil-applied fungicides at some point during the production cycle. Continued detections of the plant pathogenic fungus *Phytophthora ramorum* S. Werres, A.W.A.M. de Cock on ornamental nursery stock in North America and the potential spread of this organism throughout the nursery industry (Hansen et al. 2005; Dart et al. 2007), as well as the discovery that this organism has a well developed soil phase (Dart et al. 2007; Shishkoff 2007), has lead to an increased use of soil-applied fungicides. As a result, an understanding of the potential impact of fungicides on *M. anisopliae* is critical to the successful integration of this new microbial control agent for BVW into the nursery production system.

There have been a number of studies performed to determine the in vitro impact of agro-chemicals (fungicides, herbicides, insecticides and acaricides) on a wide variety of entomopathogenic fungi (Loria et al. 1983; Li and Holdom 1994; Todorova et al. 1998; Batista Filho et al. 2001; Wei et al. 2004; Mochi et al. 2005, 2006; Klingen and Westrum 2007; Luz et al. 2007). Moorhouse et al. (1992) performed a

detailed study of both the in vitro and in situ effects of fungicides and insecticides on *M. anisopliae* (275–86) for BVW control. The authors found that laboratory based screenings alone were limited in their ability to assess the compatibility of chemicals with *M. anisopliae*. Differences in chemical compatibility may also differ between isolates of the same fungus (Olmert and Kenneth 1974; Er and Gökçe 2004). Therefore, determining the compatibility of the commercially available *M. anisopliae* isolate with soil-applied fungicides is of critical importance. It is also important to investigate how these fungicides affect the rhizosphere competence of the fungus and to my knowledge no such studies have been conducted to date.

The objectives of these studies were to determine the impact of 17 fungicides labeled for soil application to ornamental nursery plants in the laboratory on *M. anisopliae* conidial germination and inhibition of mycelial growth. In addition, the impact of fungicide application to *M. anisopliae* population in soilless potting media (bulk and rhizosphere soil) was also determined.

Materials and methods

Fungus

The commercial isolate of *M. anisopliae* (F52) (Novozymes Biologicals Inc., Salem, VA) was used. This product consists of a sporulated and dried culture of *M. anisopliae* on rice with a concentration of 1.7×10^9 spores/g formulated product. Pure cultures for use in germination and mycelial growth inhibition experiments were grown on potato dextrose agar (PDA) at 28°C in complete darkness for 14 days prior to use. Spore suspensions were prepared by flooding sporulating cultures with 10 ml of sterile 0.1% Tween 80 solution and agitating with a sterile loop. A hemocytometer was used to adjust spore concentrations to 1×10^6 spores ml^{-1}. For the in situ fungicide applications, the formulated product was incorporated at the manufacturers recommended rate of 0.30 kg m^{-3} into a 2:1 mixture of peat moss (Sunshine Mix #3, Sun Gro Horticulture, Bellevue, WA) and perlite (Supreme Perlite Co., Portland, OR) for subsequent fungicide application.

Fungicides

Table 1 lists the manufacturer, trade name, active ingredient, formulation, reapplication interval and rate tested of the fungicides used in these studies.

Germination in vitro

Sabouraud dextrose agar + 1% yeast extract (SDAY) was prepared at double the desired final concentration, cooled to 50°C and thoroughly mixed at a 1:1 ratio with each of 17 fungicides (see Table 1) suspended in sterile water at the median recommended rate for each compound when applied as a soil drench. Control plates consisted of 1:1 ratio of SDAY agar and water. Twenty milliliters of each mixture were poured into 15 × 100 mm Petri plates and allowed to solidify. One hundred microliters of a 1×10^6 suspension of *M. anisopliae* spores were spread over the surface of Petri dishes containing the mixture of SDAY and fungicides. Petri plates were incubated in the dark for 24 h at 28°C. The percentage germination was assessed by randomly observing 200 spores. Spores were considered germinated if their germ tube was twice as long as the spore. Each treatment was replicated five times. The entire assay was performed twice.

Inhibition of mycelial growth

Petri plates of SDAY and fungicides were prepared as described above. A small plug (1 mm deep and 11.5 mm in diameter) of unsporulated mycelium from five days old culture of *M. anisopliae* was placed in the center of Petri dishes containing the fungicide incorporated SDAY. Petri plates were incubated in the dark for four days at 28°C. The radial growth (beyond the 11.5 mm diameter of the plug) was measured with digital micro-calipers to the nearest 0.01 mm in each cardinal direction drawn previously on the base of each Petri plate and recorded. Each treatment was replicated five times. The entire assay was performed twice.

Soil drench

Factorial experiments were arranged in a randomized complete block design with five replications. There were 18 containers (#1 [3.8 l], Anderson Die and

Table 1 Fungicides tested for their compatibility with *Metarhizium anisopliae* (F52)

Active ingredient	Trade name	Formulation	Rate (g or ml l^{-1})	Reapplication interval (days)[a]	Manufacturer
Azoxystrobin	Heritage	WG	0.05	21	Syngenta Crop Protection
Captan	Captan 50WP	WP	2.4	7	Agway Inc
Dimethomorph	Stature DM	WP	0.4	14	BASF Corp
Etridiazole	Terrazole 35WP	WP	0.4	28	Chemtura Corp
Fludiox + mefanox	Hurricane	WP	0.1	28	Syngenta Professional Prod
Fludioxanil	Medallion	WP	0.1	28	Syngenta Professional Prod
Fosetyl-AI	Alliette	WP	0.7	28	Bayer Crop Science
Iprodione	Iprodione Pro 2SE	SE	1.0	14	BASF Corp
Mafanoxam	Subdue MAXX	MC	0.1	28	Syngenta Professional Prod
Phosphorus acid/K-salts	Agri-Fos	EC	0.7	28	Agrichem
Propamocard	Banol	WP	2.0	14	Bayer Crop Science
Pyraclostrobin	Insignia	WG	0.6	21	BASF Corp
Quintozene	Terraclor 75WP	WP	0.5	28	Chemtura Corp
Thiophanate-methyl	Cleary's 3336F	F	0.9	21	Cleary Chemical
Thiophanate-methyl	Banrot 40WP	WP	0.5	28	Scotts Sierra Crop Protection
Triflozystrobin	Compass	WG	0.1	28	Bayer Environmental Science
Triflumizole	Terraguard 50WP	WP	0.3	14	Chemtura Corp

EC emulsifible concentrate, F flowable, MC microemulsion concentrate, SC suspension concentrate, SE suspoemulsion, WD water-dispensable granule, WP wettable powder

[a] All fungicides applied twice

Mfg. Co., Portland, OR 97222) in each replicate containing potting media incorporated with the formulated *M. anisopliae* based fungal product (Met52, Novozymes Biologicals Inc., Salem, VA) at the recommended rate of 0.30 kg m^{-3}. This corresponds to approximately 10^6 conidia/g dry potting media. This rate of fungal inoculum is efficacious against BVW for up to two growing seasons (Bruck and Donahue 2007). Replications were prepared separately using a concrete mixer (ran for 10 min) to uniformly incorporate the *M. anisopliae* spores into the potting media. Rooted cuttings of *Picea abies* (L.) Karst. (Pinales: Pinaceae) 'Nidiformis' were potted into all containers and maintained in a greenhouse at 21°C. Containers were drenched with their respective fungicide twice. The first drench was applied three weeks after potting, and the second 7–28 days after the first application depending on the prescription for each fungicide. Thirty days following the second fungicide application for each treatment, bulk media and rhizosphere soil were sampled following the procedure described by Bruck (2005). Briefly, 10 g of bulk potting media were placed in a plastic 250 ml Erlenmeyer flask containing 90 ml of 0.05% Tween 80 solution, shaken (250 rpm) for 20 min at room temperature, then placed in an ultrasonic cleaner (Model 5210, Branson Ultrasonic Corp., Danbury, CT) for two min. Serial dilutions were plated using a spiral plater (iUL Instruments, Barcelona, Spain) onto two replicate plates of media selective for *M. anisopliae* (Veen and Ferron 1966). Plates were incubated in complete darkness at 28°C for four days. The number of CFU/g dry bulk media were averaged across replicate plates for each sample. To quantify the fungal population in the rhizosphere, plants were shaken gently until only media tightly adhering to the root remained (rhizosphere). Plants were cut at their bases and the above ground portion discarded. To quantify the *M. anisopliae* population in the rhizosphere, the entire root system from each plant was placed into a plastic 250 ml Erlenmeyer flask containing 90 ml of 0.05% Tween 80 solution and processed as above. To quantify the amount of rhizosphere media on the root system of each plant sampled, the remaining suspension in each flask (once the roots were removed) was poured into a

pre-weighed aluminum pan. Each flask was carefully flushed with distilled water to remove all soil particles. Pans containing the suspension were placed in a 38°C drying oven until dry (approximately 24 h) and weighed.

Statistical analysis

Data from the spore germination and mycelial growth experiments were analyzed using the general linear models procedure (GLM) with Tukey's multiple range test used to separate means (SAS Institute 1999). An arcsine transformation of the percentage spore germination was performed to stabilize variance (Snedecor and Cochran 1989). A test of homogeneity of variance was performed to detect variation between the two runs of each experiment (Little and Hills 1978). Variability was not significantly different between runs of either experiment (germination or mycelial growth) and the data from the two runs were combined for analysis.

Analysis of the fungal population data from the bulk and rhizosphere media sampling was performed using (GLM) with Tukey's multiple range test used to separate means (SAS Institute 1999). The data from each of the four reapplication intervals (7, 14, 21 and 28 days) were analyzed separately and means separation performed within each. A one sample t−test was performed to determine if the difference between the $\log_{10}$ CFU/g dry potting media in the rhizosphere and bulk media of each plant sampled was significantly different from zero. The t−test revealed that for each fungicide, the difference between the mean $\log_{10}$ CFU/g dry soil (colony forming units) in the rhizosphere and bulk media was not significantly greater than zero ($P > 0.05$).

Results

In vitro germination and growth

There were a number of fungicides in vitro which significantly reduced the germination ($df = 17, 161$; $F = 93.70$; $P < 0.001$) and mycelial growth ($df = 17, 326$; $F = 152.04$; $P < 0.001$) of *M. anisopliae*. Because I was most interested in the impact that each fungicide had on *M. anisopliae*, the effect of each treatment on spore germination and mycelial growth is only being reported relative to the control and not between fungicides. The fungicides thiophanate-methyl, dimethomorph, captan, triflumizole, triflozystrobin, pyraclostrobin, azoxystrobin significantly inhibited spore germination relative to the untreated control (Table 2, $P < 0.0001$). Reduction in mycelial growth was even more widespread among fungicide treatments. All of the fungicides which inhibited spore germination also significantly reduced mycelial growth. In addition, the fungicides fosetyl-AI, quintozene, fludioxanil, fludiox + mefanox, iprodione, phosphorus acid/K-salts also significantly inhibited mycelial growth relative to the control (Table 2, $P < 0.0001$). Only three fungicides (etridiazole, propamocard and mafanoxam) had no significant impact on either spore germination or mycelial growth in vitro relative to the control (Table 2).

Fungicide drench applications

The fungicidal impact of the products tested was significantly less pronounced when applied as a topical drench to potting media incorporated with *M. anisopliae*. None of the chemicals tested had any significant impact on the number of CFU/g dry soil remaining in the bulk soil 30 days after the second fungicide application relative to the untreated control at any reapplication interval (Table 3). However, there was a significant reduction in the number of CFU/g dry rhizosphere soil in treatments receiving captan and triflumizole with 7 and 14 days reapplication intervals, respectively (Table 3). There was also a reduction in the number of CFU/g dry rhizosphere soil in the treatment receiving iprodione although not statistically different from the 14 days reapplication interval control; the mean value was also not significantly different from the triflumizole treatment (Table 3). A t−test revealed that for each fungicide, the difference between the mean $\log_{10}$ CFU/g dry soil in the rhizosphere and bulk media was not significantly greater than zero ($P > 0.05$).

Discussion

A number of soil fungicides labeled for use as a drench application on ornamental nursery plants had an adverse effect on *M. anisopliae* (F52) germination and mycelial growth in vitro. Mycelial growth of

Table 2 Impact of 17 fungicides registered for soil application in container-grown ornamentals on *M. anisopliae* (F52) mean (SD) spore germination and mycelial growth in vitro

Active ingredient[a]	Germination[b]	Mycelial growth (mm)[b]
Azoxystrobin	31.01 (17.75)*	1.12 (0.51)*
Captan	0.00 (0.00)*	4.51 (1.09)*
Dimethomorph	0.00 (0.00)*	3.06 (1.03)*
Etridiazole	95.33 (8.95)	13.22 (0.91)
Fludiox + mefanox	86.85 (7.02)	5.54 (1.18)*
Fludioxanil	92.95 (3.72)	5.09 (1.04)*
Fosetyl-AI	95.33 (9.05)	9.56 (4.50)*
Iprodione	95.45 (3.80)	1.18 (0.80)*
Mafanoxam	96.27 (6.29)	12.96 (1.53)
Phosphorus acid/K-salts	95.40 (11.02)	9.84 (4.37)*
Propamocard	95.59 (5.81)	14.83 (1.34)
Pyraclostrobin	0.00 (0.00)*	0.85 (0.58)*
Quintozene	97.66 (5.39)	6.50 (0.83)*
Thiophanate-methyl[c]	80.29 (7.94)*	0.44 (0.79)*
Thiophanate-methyl[d]	71.38 (15.39)*	1.12 (0.76)*
Triflozystrobin	81.03 (12.47)*	2.03 (0.83)*
Triflumizole	26.36 (20.21)*	0.93 (0.43)*
Control	94.27 (8.40)	13.63 (1.06)

[a] See Table 1 for detailed information on each fungicide

[b] Mean percentage spore germination or mm mycelial growth denoted with an (*) indicate that the value in the same column was significantly less than the untreated control ($P < 0.05$, general linear model procedure, SAS Institute 1999)

[c] Cleary's 3336

[d] Banrot 40WP

M. anisopliae was more sensitive to fungicides than spore germination. Moorhouse et al. (1992) also found the impact of several fungicides and pesticides more pronounced on mycelial growth than spore germination of *M. anisopliae*. Negative impacts of fungicides in vitro on entomopathogenic fungi are widespread. Several fungicides completely inhibited the conidial germination of two *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Deuteromycota: Hyphomycetes) isolates at recommended rates, while captan and dichlofluanid completely inhibited germination of both isolates at one tenth the recommended rate. All fungicides tested completely inhibited germination of both isolates at 10× recommended rates (Er and Gökçe 2004). Captan and dichlofluanid also completely restricted mycelial growth of both *P. fumosoroseus* isolates. Pirimicarb was the only fungicide tested at recommended or reduced rates that did not significantly inhibit mycelial growth of *P. fumosoroseus* (Er and Gökçe 2004). Fungicides were more damaging than methiocarb (acaricide, insecticide, molluscicide) in strawberry production on *Neozygites floridana* (Weiser and Muma) Remaudière and S. Keller (Zygomycetes: Entomopthorales) survival and efficacy for control of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) (Klingen and Westrum 2007).

The impact of applied soil fungicides to *M. anisopliae* (F52) spores incorporated into soilless potting media was much less profound than observations in vitro. Studies in which the impact of fungicides in the field was determined in addition to in vitro effects highlights this fact. Klingen and Haukeland (2006) provide a comprehensive review of the effects of agrochemicals on entomopathogenic fungi and concluded that insecticides and herbicides were not generally harmful to fungal growth, while fungicides were sometimes harmful. However, most of the studies cited were performed in vitro and extrapolation of laboratory results to the field are difficult to make. None of the fungicides tested in these studies had any significant impact of the number of CFU in bulk soil. This was true even for the fungicides captan and triflumizole both of which had reapplication intervals of 14 days or less and significantly reduced germination and mycelial growth in vitro. It was expected that application of these chemicals which were fungicidal (inhibiting germination of fungal spores) and fungistatic (retarding development of mycelia while in contact with the chemical) in vitro applied repeatedly in a short period of time would result in reduced fungal populations. This was not the case for fungal populations in the bulk soil. Moorhouse et al. (1992) also found little correlation between in vitro laboratory studies and in situ applications of fungicides and insecticides for *M. anisopliae*. In their studies, there were some indications that reduced germination in vitro may be linked to reduced infection rates in soil, but this relationship was not significant. Chandler and Davidson (2005) also found *M. anisopliae* to be compatible with iprodione and tebuconazole under glass house conditions for the control of *Delia radicum* (L.) (Diptera: Anthomyiidae) even though these fungicides were inhibitory to fungal growth in vitro. Iprodione was also inhibitory to fungal growth in this study while having no adverse effect on CFU numbers

Table 3 Impact of 17 fungicides registered for soil application in container-grown ornamentals on *M. anisopliae* (F52) mean (SD) colony forming units in peat-based potting media and the rhizosphere of *Picea abies* 'Nidiformis'

Active ingredient[a]	$\log_{10}$ CFU[b]	
	Rhizosphere soil	Bulk soil
7 Days ($df = 1, 8$; $F = 8.51$, $P = 0.009$; $F = 0.71$, $P = 0.41$)[c]		
Captan	5.84 (0.14)b	6.10 (0.16)a
Control	6.25 (0.43)a	6.18 (0.26)a
14 Days ($df = 4, 45$; $F = 4.80$, $P = 0.003$; $F = 0.56$, $P = 0.70$)		
Dimethomorph	6.11 (0.20)a	6.17 (0.33)a
Iprodione	5.98 (0.21)ab	6.19 (0.22)a
Propamocard	6.15 (0.16)a	6.26 (0.26)a
Triflumizole	5.84 (0.19)b	6.11 (0.18)a
Control	6.00 (0.10)a	6.08 (0.44)a
21 Days ($df = 3, 36$; $F = 0.56$, $P = 0.64$; $F = 0.22$, $P = 0.88$)		
Azoxystrobin	6.01 (0.29)a	6.18 (0.25)a
Pyraclostrobin	5.91 (0.15)a	6.15 (0.22)a
Thiophanate-methyl[d]	6.02 (0.20)a	6.24 (0.34)a
Control	5.95 (0.15)a	6.18 (0.18)a
28 Days ($df = 10, 99$; $F = 0.90$, $P = 0.53$; $F = 1.72$, $P = 0.09$)		
Etridiazole	6.09 (0.25)a	6.13 (0.20)a
Fludiox + mefanox	6.04 (0.35)a	6.10 (0.29)a
Fludioxanil	6.00 (0.14)a	6.16 (0.31)a
Fosetyl-AI	6.04 (0.26)a	6.23 (0.21)a
Mafanoxam	6.21 (0.31)a	6.18 (0.23)a
Phosphorus acid/K-salts	5.86 (0.17)a	5.99 (0.27)a
Quintozene	5.95 (0.24)a	6.22 (0.21)a
Thiophanate-methyl[e]	6.04 (0.40)a	6.18 (0.13)a
Triflozystrobin	5.98 (0.32)a	6.21 (0.17)a
Control	6.02 (0.38)a	5.89 (0.36)a

[a] See Table 1 for detailed information on each fungicide. Interval between initial and second fungicide application

[b] Mean $\log_{10}$ colony forming units. The fungal population was determined 30 days after the second fungicide application. Means in the same column with the same reapplication interval denoted by different letters are significantly different ($P < 0.05$, general linear model procedure, SAS Institute 1999)

[c] Degrees of freedom from the general linear models procedure performed for each reapplication interval followed by the F and P value from the $\log_{10}$ colony forming units analysis of the rhizosphere and bulk soil fungal populations, respectively

[d] Cleary's 3336

[e] Banrot 40WP

when applied to potting media incorporated with *M. anisopliae* spores. Fungicides used in sugarcane production (prochloraz, propiconazole, flusilazole and methyl ethyl mercuric chloride) also significantly inhibited mycelial growth of *M. anisopliae* in vitro, but were found be compatible in commercial practice (Samson et al. 2005). The activity of *M. anisopliae* against *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) was significantly reduced when the fungicides chlorothalonyl and tebuconazol were applied to soil. This was not the case in the acaricide, insecticide and herbicide treatment (Mochi et al. 2006). Based on fungal respiratory activity, the toxic action of a range of pesticides (acaricides, fungicides, insecticides and herbicides) on *M. anisopliae* in the soil is small, suggesting little negative impact on the fungal activity resulting from their use (Mochi et al. 2005).

In the rhizosphere soil, some fungicides tested in these studies significantly reduced the number of *M. anisopliae* CFU. To my knowledge, this is the first study to consider the impact of fungicides on entomopathogenic fungal populations in the rhizosphere. The biology of entomopathogenic fungi outside of their role as entomopathogens is becoming an increasingly important area of study. The potential of utilizing rhizosphere competent entomopathogenic fungi is great and any adverse effect that chemical fungicides have on the fungal population in the rhizosphere must be considered. If the fungus is not able to persist and proliferate in the rhizosphere it may not be present at levels adequate to control BVW larvae feeding on the roots. There was no significant increase in the *M. anisopliae* populations in the rhizosphere in this study relative to the bulk soil populations as was observed by Bruck (2005). It can take as long as 8–10 weeks for fungal populations to increase significantly in the rhizosphere (unpublished data). It appears that the duration of this study was to short for *M. anisopliae* to become established and proliferate in the rhizosphere. The fungicides (captan and triflumizole) which significantly reduced fungal populations in the rhizosphere were fungistatic and fungicidal in vitro and had short (7–14 days) reapplication intervals. The impact of other chemicals has been shown to reduce entomopathogen infection rates when applied with short reapplication intervals (Hall 1981; Anderson and Roberts 1983). There were a number of other fungicides (thiophanate-methyl, triflozystrobin, pyraclostrobin and azoxystrobin) with similar qualities in vitro with longer reapplication intervals (>14 days) that had no significant impact on fungal populations in the rhizosphere in situ. Iprodione was fungistatic in vitro and while it did not significantly reduce fungal populations in the rhizosphere, the resulting fungal population was also not significantly different than those for triflumizole. Propamocard and dimethomorph were the only fungicide tested with a 7–14 days reapplication interval that did not have any effect on *M. anisopliae* populations in situ. Propamocard was also the only fungicide with a 7–14 days reapplication interval that had no impact in vitro on *M. anisopliae*. The results of the in vitro bioassays of fungicides with <14 days reapplication intervals were somewhat predictive of the impact that these fungicides had on *M. anisopliae* populations in the rhizosphere.

The use of *M. anisopliae* in the rhizosphere is a potentially new approach for BVW management in container-grown ornamentals that may be negatively impacted by some fungicides labeled for use in the industry. It appears from theses studies that the impact is limited to fungicides with fungicidal and fungistatic effects in vitro and reapplication intervals of less than 14 days. Fungicides with similar effects in vitro and reapplication intervals >14 days were not detrimental to rhizosphere populations, presumably due to the length of time between fungicide applications. It may be that reapplication intervals of greater than 14 days are adequate for the fungal population in the rhizosphere to rebound. In soils not treated with fungicides, *P. abies* can support rhizosphere populations of *M. anisopliae* (F52) up to 10× the level in surrounding bulk soil (Bruck 2005).

Metarhizium anisopliae (F52) clearly has a great deal of potential as a microbial control agent for BVW (Bruck 2005, 2006, 2007; Bruck and Donahue 2007). *M. anisopliae* is currently labeled for use as a soil incorporant, and when used as such is compatible with all of the fungicides tested. As researchers develop and growers begin to implement rhizosphere competence as an alternative management strategy for BVW, the fungicides captan and triflumizole should be avoided.

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